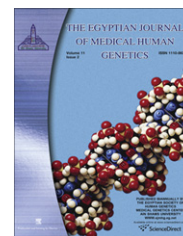




Ain Shams University

The Egyptian Journal of Medical Human Genetics

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ORIGINAL ARTICLE

Hair mercury measurement in Egyptian autistic children

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Received 10 July 2009; accepted 1 October 2009

KEYWORDS

Autism;
 Mercury;
 Hair analysis;
 Toxic metals

Abstract *Background:* A review of medical literature has shown that exposure to mercury, whether organic or inorganic, can give rise to the symptoms and traits defining or commonly found in autism spectrum disorders (ASD). Mercury can cause impairments in social interaction, communication difficulties, and repetitive and stereotyped patterns of behavior, which comprise the three DSM-IV diagnostic criteria of autism. The aim of this work was to measure the concentration of total mercury trace elements in the hair of some Egyptian autistic children and to correlate these levels with severity of the disease.

Methods: Thirty- two patients diagnosed by DSM-IV-TR criteria (diagnostic and statistical manual of mental disorders, 4th edition criteria, text revised) were subjected to hair mercury measurement using Atomic Absorption Spectrometry (AAS) and were compared to hair mercury measurement of fifteen, age and sex matched healthy children.

Results: Results revealed a highly significant increase in the mean hair mercury level in autistic patients than the control group (0.79 ± 0.51 vs 0.12 ± 0.086 ppm) respectively, ($P < 0.001$). There

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was a significant increase of mercury level in autistic children who received routine and additional vaccines, and there was mild yet not significant increase in mercury level in patients with maternal history of dental amalgam and high fish consumption during pregnancy and also in autistic children whose mother received anti-D.

Conclusion: There was a higher concentration of mercury levels in the hair of children with autism as compared to the age and sex matched healthy controls. Hair analysis is of potential usefulness for determination of mercury level and offering a chance for intervention to treat by chelation therapy.

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1. Introduction

Autism spectrum disorders encompass a spectrum of developmental disorders characterized by impairment in several behavior domains. There is usually impairment in the development of language, communications and reciprocal social interaction, together with a restricted repertoire. Onset is typically before age of 3 years [1]. While genetic factors are clearly important, as indicated by high concordance rates among twins and siblings, they alone cannot account for an epidemic that developed in the relatively short period of 10–20 years [2]. Thus environmental factors are very likely to account for the major portion of the increased prevalence of autism. Exposure to xenobiotics is an inevitable feature of contemporary life driven by an ever increasing number of threatening chemicals found in air, water and food supplies and other materials we come in contact with during our daily routine [3].

Heavy metals, such as arsenic, lead and mercury, listed as the three highest priority hazardous substances by the US Department of Health and Human Services is of particularly high concern, since even low levels are associated with neurological impairments, including attention-deficit hyperactivity disorder (ADHD) and lower IQ. Other heavy metals (cadmium, antimony, manganese, nickel, etc.) exert similar effect [4]. Mercury is a well-known neurotoxin. There are three kinds of mercury exposure: elemental mercury poisoning, inorganic mercury poisoning and organic mercury poisoning. Organic mercury is the most toxic [5]. A review of medical literature has shown that exposure to mercury, whether organic or inorganic, can give rise to the symptoms and traits defining or commonly found in autism spectrum disorders (ASD) individuals [6]. Mercury can cause impairments in social interaction, communication difficulties, and repetitive and stereotyped patterns of behavior, which comprise the three DSM-IV autism diagnostic criteria. Additionally, mercury can induce features prominent in ASD, such as sensory abnormalities, emotional/psychological changes, movement disorder, impairments in abstract or complex thinking, severe sleep disturbances, and self injurious behavior. Males are more affected than females in both conditions [7]. The disease characteristics that suggest this possibility are: (a) ASD traits are known to arise from mercury exposure; (b) Onset of ASD symptoms is associated with administration of immunizations; (c) The reported increase in the prevalence of autism in the 1990s closely follows the introduction of two mercury containing vaccines; and (d) Elevated mercury has been detected in biological samples of autistic patients [7]. Studies have shown that there is a biological possibility and epidemiological evidence showing a direct relationship between increasing doses

of mercury from thimerosal-containing vaccines and neuro-developmental disorders [8].

The concentrations of trace elements in hair from normal children differ from patterns observed in both autistic and autistic-like children. Furthermore, some studies, suggested that mercury hair analysis may have potential use as a diagnostic tool for autism [9].

The aim of this work was to measure the concentration of total mercury trace elements in the hair of Egyptian autistic children and to correlate these levels with disease severity.

2. Patients and methods

This case-control study was conducted on thirty-two patients diagnosed with autism based on DSM-IV-TR criteria (American psychiatric association, 1994 diagnostic and statistical manual of mental disorders, 4th edition criteria, text revised) [10,11]. They were followed up at Psychiatry Clinic, Children Hospital, Ain Shams University. The patients were twenty-two males and ten females, their ages ranged from 2 to 13 years (mean age 6.75, SD \pm 3.26 years).

Exclusion criteria include coexisting medical disorders related to autism as tuberous sclerosis and neurofibromatosis.

The control group included 15 healthy properly matched children in age, sex, environment and habitat. They were nine males and six females. Their ages ranged from 2 to 11 years (mean age 5.53, SD \pm 2.75 years), some of them were siblings of autistic patients.

All cases were subjected to the following

I- Detailed history taking with special emphasis on:

- Onset, course and duration of the disease.
- Antenatal or maternal history: maternal age at birth, parity, any fetal loss, chronic illness, infections or hospitalizations during pregnancy, medications (e.g. antiepileptic drugs, anti-thyroid drugs), dietary supplements (the type and amount of fish consumption by the mother during pregnancy), dental work (filling amalgam or removal), anti-D immunoglobulins given during pregnancy, occupation, cigarette, alcohol, or substance abuse during pregnancy.
- Natal and postnatal history.
- Developmental history (both mental and motor).
- Past history including major childhood illnesses, injuries, diet, medication, immunizations.
- Environmental exposure: home environment, water source, gasoline station, or dry cleaner in close proximity to the child's home. Potential environmental exposures in the neighborhood, broken thermostats or thermometers.

- Family history of similar condition or any psychological or mental disorders.
- II- Thorough clinical examination laying stress on neurological examination.
- III- Psychiatric evaluation:
- Confirmation of diagnosis using DSM-IV-TR criteria of autism. i.e. impairments of language, social skills, and restricted stereotyped interest or activity.
- Assessment of mental age using Stanford–Binet intelligence scale (1986) [12], to calculate the intelligence quotient (IQ). This test is used to measure the child cognitive abilities. It is suitable for children aging from 2–16 years. The test has two items, the verbal and the performance and the test item is chosen according to the child abilities. IQ was calculated by dividing the mental age by the chronological age multiplied by 100. Subnormal intellectual function is diagnosed when IQ is below 70.
- Assessment of severity of autistic symptoms using childhood autism rating scale (CARS) [13] which rates the child on a scale from one to four in each of fifteen areas (relating to people, emotional response, imitation, body use, object use, listening response, fear or nervousness, verbal communication, non-verbal communication, activity level, level and consistency of intellectual response, adaptation to change, visual response, taste, smell, touch response and general impression).
- IV- Measurement of mercury levels in the hair of the children:

Hair sampling is a non invasive technique, it is the best indicator of a given mineral in the body.

2.1. Hair specimen collection

These samples were collected from cases and control by single cutting from the occipital region. The samples were cut to lengths of about 1.5–2 cm using clean stainless steel scissors. A minimum of 5–10 mg of hair was required for the hair analysis assay. Approximately 100 strands of hair (~50 mg) were used. Adhesive paper was placed over the end of the hair strands closest to the scalp; the paper was marked with an arrow indicating the end of hair closest to the scalp. The samples were placed in a sealed plastic bag [14].

2.2. Hair Hg analysis

This was done in our study using Atomic Absorption Spectrometry (AAS)/hydride system which is one of the most sensitive analytical techniques used for trace element determination. The determination depends on the formation of atomic mercury at room temperature after reacting with strong reducing agent as tin (II) chloride (stannous chloride) SnCl_2 or Sodium borohydride is used to liberate Hg as follows:



2.3. Mercury reduction

- All mercury must be in ionic form, most is present in Organic-Mercury Complexes.

- Mercury solutions are unstable, 0.01% $\text{K}_2\text{Cr}_2\text{O}_7$ in 0.8 M HNO_3 is used to prevent mercury loss.

2.4. Methodology

2.4.1. Sample preparation

- The samples were cleaned by acetone three times then washed by ultra pure water and dried in an oven at 70 °C over night. The hair samples were then digested for 15 min by adding 4 ml of HNO_3 and 2 mL of H_2O_2 , to 0.1 g hair. The digested samples were introduced to Hydride system AAS for mercury analysis [15].
- N.B. The study was approved by the ethics committee of faculty of medicine, Ain shams university. A written informed consent was obtained from parents of all children involved in the study.

2.4.2. Statistical methodology

Data entry and analyses were performed using SPSS statistical package version 10 (SPSS Inc., Chicago, IL, USA). The data were examined for normal distribution using Kolmogorov–Smirnov test. Mean, standard deviation, median and range were calculated for quantitative data. Qualitative data were presented as number and percent and the association between column and raw variables were examined using chi-square (χ^2) test.

Student *t*-test was used to compare means of two groups. Mann Whitney-*U* test and Kruskal–Wallis *H* are non-parametric tests equivalent of the *t*-test and ANOVA test respectively. Correlation between variables was done using Spearman rank correlation for non-parametric data.

For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (*P* value). *P* value of <0.05 indicates a significant result while, *P* value of <0.001 indicates a high significant result.

Box plots are useful to display differences between populations without making any assumptions of the underlying statistical distribution: they are non-parametric. The spacing between the different parts of the box helps to indicate the degree of dispersion (spread) and skewness in the data. Box plots can be drawn either horizontally or vertically five-number summaries:

- The smallest observation (sample minimum).
- Lower quartile (Q1) = 25% of our study observation.
- Median (Q2) = 50% of our study observation.
- Upper quartile (Q3) = 75% of our study observation.
- Largest observation (sample maximum) [16].

3. Results

This case-control study included thirty-two patients with autistic spectrum disorders (ASDs). They were 22 males (68.6%) and ten females (31.3%) with a male to female ratio 2:1, their ages ranged from 2–13 years with a mean age 6.7, SD ± 3.2 years, eleven (34.4%) were less than 5 years, 14 (43.8%) from 5–9 years, seven (21.9%) were >9 years Table 1. The control group comprised fifteen healthy properly age and sex matched children, nine males and six females, their ages

Table 1 Demographic data of the patients and frequency of mercury exposure.

Age of patients	
< 5 years	11 (34%)
5–9 years	14 (44%)
> 9 years	7 (22%)
Age of diagnosis	
1–3 years	29 (90.6%)
3–6 years	3 (9.4%)
Sex M/F	22/10 (68.6% vs 31%)
Clinical types of ASD: AD/AS/Rett	28/3/1
Frequency of mercury exposure	
Maternal dental amalgam	5 (15.6%)
Anti-D	3 (9.4%)
Increased maternal fish consumption during pregnancy	4 (12.5%)
Routine vaccination	32 (100%)
Additional vaccination	11 (34.4%)

ASD: autism spectrum disorder, AD: autism, AS: Asperger syndrome.

ranged from 2–11 years; (mean 5.5, SD ± 2.7 years), none of them had a history of chronic illness. Twenty-nine patients (90.6%) were diagnosed before the age of 3 years while three patients (9.4%) were diagnosed after 3 years. Twenty-eight pa-

tients (87.5%) had typical autism (AD), three patients (9.4%) had Asperger syndrome and one patient (3.1%) had Rett syndrome. As regards to frequency of exposure to mercury, five patients (15.6%) had history of maternal dental amalgam, three patients (9.4%) had Rh –ve mothers who received Anti-D immunoglobulin, four patients (12.5%) had history of increased maternal fish consumption during pregnancy (three times per week), all patients received routine vaccination, and only eleven patients (34.4%) had additional vaccination (influenza, chicken pox, meningitis) **Table 1**.

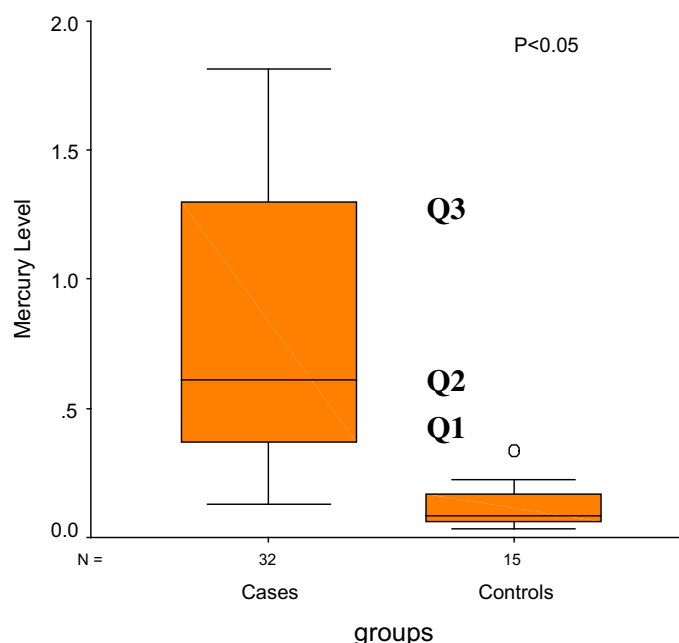
There was a highly statistically significant difference between mean hair mercury level in cases (0.79 ± 0.51 ppm) and mean mercury level in control group (0.12 ± 0.09 ppm) ($P < 0.001$) **Table 2**, **Fig. 1**. There was a significant increase in hair mercury level in autistic patients who received routine and additional vaccination such as (influenza, chicken pox and meningitis vaccine) **Fig. 2**. There was a mild yet not significant increase of mean hair mercury level in autistic patients with history of maternal dental amalgam and fish consumption during pregnancy and in patients whose mother received anti-D immunoglobulin (**Table 3**).

There was no significant difference in mercury level in different IQ groups of patients, however, mercury level was higher in the group of mentally retarded patients. There was no significant difference in mercury level in different age groups of patients. The mean mercury level in male patients was high-

Table 2 Statistical comparison of mean hair mercury levels in cases and control group.

Mercury (Hg) level	Control ($n = 15$)	Cases ($n = 32$)	Mann–Whitney test	
Mean (PPM)	0.121	0.794	Z	P
\pm SD	0.086	0.513	–5.18	0.001**
Range	0.04–0.34	0.13–1.81		
Median	0.081	0.611		

** Highly significant $P < 0.001$.

**Figure 1** Modified box plots show the median of mercury levels in cases and control.

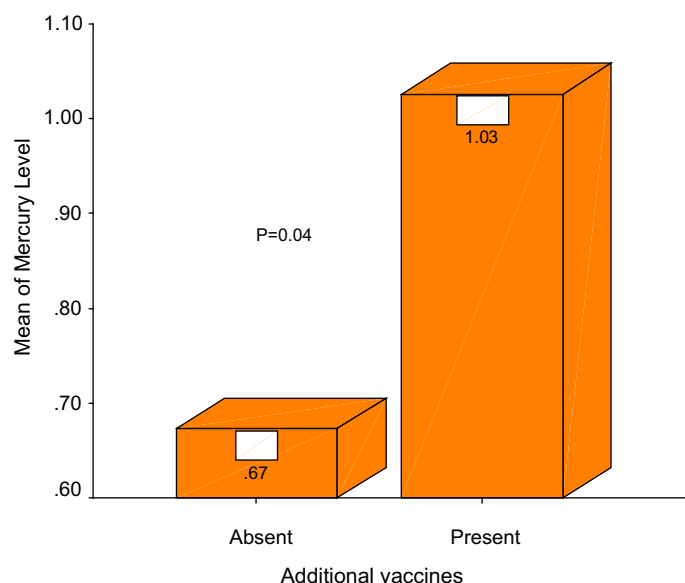


Figure 2 Histogram shows the mean Hg levels in cases according to history of additional vaccines.

Table 3 Statistical comparison of mean hair mercury levels in cases as regards mercury exposure.

Hg exposure	Present				Absent				Mann-Whitney test	
	Mean (PPM)	±SD	Median	Range	Mean (PPM)	±SD	Median	Range	Z	P
Dental amalgam	1.211	0.575	1.368	0.27–1.81	0.717	0.473	0.572	0.13–1.67	1.58	0.12
Anti-D	1.240	0.432	1.290	0.79–1.65	0.748	0.505	0.572	0.13–1.81	1.65	0.09
Fish consumption	1.103	0.564	1.173	0.40–1.67	0.750	0.501	0.587	0.13–1.81	1.31	0.21
Additional vaccination	1.026	0.529	0.914	0.23–1.81	0.673	0.473	0.549	0.13–1.59	2.00	0.05*

* Significant at $P < 0.05$.

er (0.85 ± 0.5 ppm) than the mean mercury level in female patients (0.68 ± 0.6 ppm) yet the difference was not statistically significant. There was no significant difference in hair mercury level between ASDs as regards to age of onset of disease or clinical presentation.

4. Discussion

Little work has been done to investigate the potential contributions of environmental neurotoxicant exposure to childhood psychiatric morbidity, although the hypothesis that autism is associated with prenatal or early postnatal exposure has been raised [17].

The current study showed a significant higher hair mercury level among autistic group compared with the age and sex matched healthy control group. This was in agreement with Fido et al., who reported that autistic children had higher levels of hair mercury than non autistic children [18].

Also our results were in accordance with other authors who hypothesized that autism spectrum disorders is a mercurial syndrome and similarities are found between prenatal /infantile mercury exposure including delayed language, defective communication and repetitive behaviors [6,19].

In contrary to our results, other studies found that hair mercury of autistic children was significantly lower than that of the

control group. They suggested that autistic children retain mercury in their body due to impairment in detoxification pathways [20,21]. However, Ip et al. found no difference in mercury levels in hair and blood of autistic children comparing with non autistic normal children [22]. The environmental protection agency (EPA) warns that pregnant women can be exposed to methylmercury by the consumption of contaminated fish or the use of dental amalgam, and their offspring may have developmental and neurological abnormalities [23].

As regard to prenatal exposure to mercury in our study, 12.5% of mothers of autistic patients consumed fish more than two times a week during pregnancy and showed increased hair mercury levels in their children. This was in agreement with many authors who reported a positive correlation between mercury concentration in hair and annual fish intake and added that fish is the major source of dietary mercury, and that cooked fish retains the same amount of mercury as raw fish [24,25].

In the present work, autistic children whose mothers had a history of dental amalgam fillings during pregnancy showed a higher level of hair mercury. This is in accordance with some authors [26,27]. Other studies found a positive correlation between fetal hair mercury level and the number of maternal amalgam fillings [28,29]. This may be due to escape of mercury vapour during the preparation of amalgam restoration and so

some of the vapour may be inhaled. However, Bellinger et al. disagreed with our results as they reported that exposure to dental amalgam restorations has no effect on neuropsychological functions or disorders [30]. Also our study revealed that 9.4% of mothers of our autistic children received anti-D immunoglobulin, and their children showed higher mercury levels. This was in agreement with other authors [20,31]. However another study opposed this finding [32]. Rodier et al. suggested that migratory cells undergoing mitosis in the neural tube of the fetus are particularly vulnerable to toxic insults. A growing fetus also lacks the important capacity for drug detoxification, and the incomplete development of the blood/brain barrier further increases vulnerability at this stage. The interactions of the natural trace elements in the body are complicated and interconnected, altering or supplementing one system may have a dramatic impact on another. Whether toxic elements are instrumental from the very beginning of the pathologic process of autism or appear later in the process and cause further damage is not known [33].

Postnatal exposure to mercury can occur by vaccination. In our study, all the autistic children received the compulsory vaccinations while only 34% of patients received additional vaccines that group showed a significant increase in hair mercury level. Also, increased cumulative doses of mercury exposure from thiomersal-containing childhood vaccines with the increasing population prevalence of children diagnosed with ASD was reported previously [34,35]. Geier et al. showed that there was a statistically significant increase in the incidence of autism and speech disorders for the thiomersal containing DTaP vaccinated cases, compared to thiomersal free DTaP vaccinated cases [36]. On the other hand, other studies concluded that there were no associations between thiomersal exposure in infant's vaccines and neurodevelopmental outcomes [37–39].

In our study, hair mercury levels of autistic patients showed no correlation with onset of disease, or different clinical presentations, however, mercury level was higher in those patients with the lowest mentality and those with severe degree of autism according to CARS, also those patients with positive family history of similar conditions or other neuro-developmental disorders.

This was in agreement with other studies that estimated a dose response relationship between maternal mercury and childhood decrements in I.Q. [40,41]. Also, mercury intoxication associated urinary porphyrins were significantly increased with the increased severity of autism as indicated by CARS score [42].

5. In conclusion

- The results of this study showed the presence of higher levels of mercury in the hair of children with autism as compared to the age and sex matched healthy controls.
- Biological damage from mercury as a neurotoxic substance, beside genetic susceptibility in the form of reduced ability to excrete mercury and/or increased environmental exposure at key times in development may play a causal role in autism.
- It is mandatory to minimize exposure to mercury through judicious consumption of fish and avoidance of dental amalgam fillings during pregnancy, as well as substitution of claimed vaccines conservatives by others that do not contain methylmercury.

- Hair analysis is of potential usefulness for determination of mercury level and offering a chance for intervention to treat by chelation therapy.
- Further research on a wider scale is needed in this area.

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